Previous studies have reported a disparity between the circumferential extent of abnormal wall thickening (WT) and that of infarct size (IS) or size of ischemic zone (IZ) during demand ischemia (DI) (1–4). The circumferential extent of abnormal WT is reported to be larger than IS or size of ischemic zone (IZ) during rest (1–3), whereas during DI the spatial extent of abnormal WT has been reported to be smaller than that of the corresponding perfusion defect (PD) (4). Although several studies have demonstrated a consistent relationship between regional myocardial blood flow (MBF) and WT within the center of infarcted or ischemic myocardium (5–7), the margins seem to behave differently. Several reasons have been postulated to explain this behavior, ranging from mechanical tethering to inherent errors in methods of regional function analysis (8,9).

We hypothesized that the apparent disparity between the circumferential extent of abnormal WT and IS during coronary occlusion or the PD size during DI is mainly due to the effects of collateral blood flow (CollBF) supplying the margins of the infarcted or ischemic tissues. Therefore, based on the well-defined flow-function relation (5–7), WT in these margins will reflect the level of MBF. Depending on the magnitude of CollBF, the extent of abnormal WT may appear to either overestimate IS or underestimate the IZ size during DI.

METHODS

Animal preparation. The protocol was approved by the animal research committee at the University of Virginia. In
18 anesthetized open-chest dogs, catheters were placed in both femoral veins for administration of drugs, fluids, and microbubbles, as well as in both femoral arteries for withdrawal of duplicate arterial blood samples for radiolabeled microsphere (RM)-derived MBF analysis. Catheters were also placed in the ascending aorta for measurement of pressure and in the left atrium for injection of RMs.

The proximal sections of the left anterior descending (LAD) and left circumflex (LCx) coronary arteries were dissected free from the surrounding tissue. Group 1 dogs (n = 6) underwent occlusion of one of these arteries, and non-critical stenoses were produced on either one (Group 2, n = 6) or both (Group 3, n = 6) of these arteries. The absence of flow in the Group 1 dogs and the presence of normal resting flow in the other two groups was confirmed by coronary flow probes.

**Echocardiography.** Echocardiography was performed using an HDI5000 system (Philips, Bothell, Massachusetts). The transducer was fixed in position, and a saline bath acted as an acoustic interface between it and the heart. The left ventricular (LV) mid-papillary muscle short-axis plane was depicted as parametric images (13) to display the spatial extent of MBF. Colors ranging from orange-brown (low flow) to white-yellow (high flow) were assigned to MBF extent of MBF. In dogs with stenosis, PD size during peak dobutamine dose was measured in the frame where there was maximal disparity in AI between beds. At this early time point (usually 1 s), normal regions were completely replenished because of hyperemia, whereas those supplied by stenosis were not. Regions that filled later (within 5 s after bubble destruction) were defined as collateral-supplied.

**RM-derived MBF analysis.** The post-mortem heart slice, corresponding to the MCE short-axis image, was cut into 16 wedge-shaped pieces. Each piece was further divided into epicardial, endocardial, and mid-circumferential portions. Parameters of mean microbubble velocity, the plateau AI representing myocardial blood volume, and the rate of rise of AI reflecting mean microbubble velocity were also depicted as parametric images (13) to display the spatial extent of MBF. Colors ranging from orange-brown (low flow) to white-yellow (high flow) were assigned to MBF values within each tissue piece. All values were normalized to the highest MBF within the short-axis slice.

**Experimental protocol.** Group 1 dogs underwent coronary occlusion for 6 h, at which time hemodynamic, WT, and MCE data were recorded and RMs were injected. In Groups 2 and 3 dogs, two to four different non-flow-limiting stenoses were produced. To define the perfusion territories of the two vessels in the Group 3 dogs, the sites of stenosis were briefly and transiently (few seconds) occluded during MCE performed at rest. A dobutamine stress protocol was performed in both Group 2 and 3 dogs (10 to 40 μg·kg⁻¹·min⁻¹ infused intravenously) (4), and at peak dobutamine dose, hemodynamic, WT, and MCE data were recorded, and RMs were injected. Wall thickening and MCE data were obtained in rapid succession 3 to 4 min after starting the peak dobutamine dose. The heart was then removed, and the short-axis slice defined by the pre-placed sutures was analyzed for IS using triphenyl tetrazolium chloride staining (14) as well as RM-derived MBF (12,13).
Table 1. Results (Normalized to the Normal Remote Bed)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 Risk Area</th>
<th></th>
<th></th>
<th>Group 2 Perfusion Defect</th>
<th></th>
<th></th>
<th>Group 3 Perfusion Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Collateralized Zone</td>
<td>Infarcted Zone</td>
<td>Collateralized Zone</td>
<td>Central Zone</td>
<td>Collateralized Zone</td>
<td>Central Zone</td>
<td></td>
</tr>
<tr>
<td>%WT</td>
<td>32 ± 23</td>
<td>6 ± 0.9</td>
<td>88 ± 9</td>
<td>64 ± 8</td>
<td>64 ± 19</td>
<td>53 ± 14</td>
<td></td>
</tr>
<tr>
<td>MBF</td>
<td>0.43 ± 0.20</td>
<td>0.23 ± 0.17</td>
<td>0.73 ± 0.17</td>
<td>0.46 ± 0.16</td>
<td>0.61 ± 0.19</td>
<td>0.53 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.55 ± 0.26</td>
<td>0.32 ± 0.15</td>
<td>0.88 ± 0.15</td>
<td>0.74 ± 0.16</td>
<td>0.78 ± 0.14</td>
<td>0.73 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>0.32 ± 0.19</td>
<td>0.09 ± 0.05</td>
<td>0.67 ± 0.17</td>
<td>0.37 ± 0.14</td>
<td>0.60 ± 0.19</td>
<td>0.51 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Aβ</td>
<td>0.25 ± 0.18</td>
<td>0.03 ± 0.02</td>
<td>0.58 ± 0.16</td>
<td>0.29 ± 0.14</td>
<td>0.48 ± 0.19</td>
<td>0.38 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

A = the plateau acoustic intensity representing myocardial blood volume; β = rate of rise of acoustic intensity reflecting mean microbubble velocity; MBF = myocardial blood flow; %WT = percent of wall thickening.

Statistical methods. Data are expressed as mean ± 1 SD. Comparisons between regions and stages in the same dogs were made using either the paired or the unpaired Student t test. Differences were considered significant at p < 0.05 (two-sided). To define the relation between %WT and MBF, a linear regression model was used. An exponential relation \( y = 57(1 - e^{-b(x - c)}) \) was defined in a previous study where 57 denotes maximal %WT, b is a constant, and c denotes MBF where %WT = 0 (6). To derive correlation coefficients, we transformed this equation to a linear form: \( \log(1 - y/57) = b(x - c) \) where r was calculated based on the transformed values of y. We also assumed independence between observations from different stages in each dog because each dog was allowed to return to baseline after each stage. However, to account for any correlation between multiple observations from different regions from the same dog, the Huber and White sandwich estimator (15,16) was used to determine the variance-covariance matrix. By this method, we assumed a “working independence model” to obtain estimates of the coefficients, and then obtained unbiased robust estimates of variances and covariances of these estimates by adjusting for the correlation between multiple observations from cluster samples. All analyses were performed using S-Plus version 2000 (Mathsoft, Inc., Seattle, Washington).

RESULTS

Group 1 dogs. The MCE-derived RA ranged from 22% to 47% (mean = 36 ± 11%), whereas the MCE-derived IS ranged from 12% to 42% of the LV short-axis slice (mean = 28 ± 12%). The CollBF-supplied zone within the RA that did not undergo necrosis ranged from 3% to 13% on MCE (mean = 8 ± 4%). The IS on tissue staining ranged from 10 to 41% (mean = 31 ± 13%). There was a good correlation (r = 0.94, p < 0.0001) between IS measured by MCE and tissue staining. The circumferential extent of abnormal WT ranged from 25% to 48% (mean = 39 ± 11%) and was significantly greater (p < 0.05) than IS measured by either MCE or tissue staining. As expected, MBF, WT, and MCE parameters were highest in the normal bed and lowest within the infarct zone. They were intermediate in the collateralized zone (Table 1).

Figure 1 illustrates data from a Group 1 dog. The circumferential extent of abnormal WT (defined as <2 SD of that in the normal bed, panel A) was almost exactly that of the RA (defined as the PD at 5 s after bubble destruction, panel B). However, the PD size at 20 s was considerably smaller (panel C) and corresponded to the patchy but transmural infarction on tissue staining (panel D). The difference in the PD sizes between panels B and C represented the CollBF-supplied region within the RA. Even though CollBF was enough to prevent necrosis, it was inadequate to preserve normal resting WT, which explains the apparent disparity between the WT abnormality and IS.

Figure 2 shows a plot of transmural MBF versus WT in Group 1 dogs with values taken from the normal, infarcted, and CollBF-supplied zones. Whereas MBF and WT were different in these three beds (Table 1), they all fell on a single line defining the WT-MBF relation, indicating that MBF and WT are closely coupled (r = 0.94). Therefore, other potential influences such as tethering do not play a significant role in the reduction in WT at the margins of the infarct.

Group 2 dogs. Figure 3 illustrates data from a Group 2 dog, five out of six of which were subjected to multiple stages. The PD size, measured at the time of maximal difference in AI between the normal and stenosis-supplied beds (1 s after bubble destruction), was large (panel A) and corresponded spatially with the region with reduced RM-derived MBF (filled arrows, panel B). The PD size was smaller 5 s later (panel C) because of filling of the lateral borders of the IZ zone by CollBF. The extent of abnormal WT (defined as <2 SD of that in the normal bed at peak dobutamine dose) was localized only to the small central region with reduced MBF (panel D), and the lateral margins of the IZ had higher MBF. Wall thickening in this collateralized region was relatively well preserved (Table 1).

Figure 4 shows a plot of MBF versus WT in these dogs. Because of dobutamine, the values for WT and MBF were high in all beds. Notwithstanding, they were different in the normal bed as well as the central and lateral portions of the IZ (Table 1). All data points again were defined by a single relationship (r = 0.70) between WT and MBF independent of the region of the myocardium from which they were derived. Because data from the CollBF-supplied regions (shown in green) lay on the plateau of this relationship, differences in WT between these and normal regions were...
relatively small. Therefore, the spatial extent of abnormal WT visually appeared to be less than that of the PD.

**Group 3 dogs.** All dogs underwent multiple (2 to 5) stages. Multiple PDs were seen in areas supplied by the stenoses during DI. In four of the six dogs, regions between the PDs exhibited the same flow-function relation described previously. These zones were supplied by vessels distal to the stenoses. The normalized %WT and MBF in these zones were $0.67 \pm 0.13\%$ and $0.63 \pm 0.15$, respectively, consistent with the absence of tethering.

In comparison, in the remaining two dogs the regions between the PDs were supplied by normal vessels and had reduced WT (normalized %WT of $0.52 \pm 0.11\%$) despite normal perfusion (normalized MBF of $0.84 \pm 0.15$), violating the flow–function relation. **Figure 5** shows examples from one of these dogs. Two PDs in regions supplied by the LAD and LCx are shown in panel A. A diagonal branch proximal to the LAD stenosis site resulted in near normal perfusion in a region between the two PDs corresponding with the RM-derived MBF data (arrows, panel B). The extent of abnormal WT (panel D), not only encompassed the regions with the PDs, but also the well perfused region in-between.

**Figure 6A** shows a plot of MBF versus WT where the values have been taken from the normal as well as different portions of the abnormal bed. The relationship is not as good as that seen in Group 1 and 2 dogs, mostly because of inclusion of small myocardial regions between PDs with relatively higher MBF (such as seen in **Fig. 5**). This finding

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**Figure 1.** Data from a Group 1 dog with left anterior descending occlusion. (A) Region of wall thickening abnormality (defined by chords and arrows). (B) Risk area (perfusion defect at 5 s after microbubble destruction). (C) Region with no flow even at 20 s after microbubble destruction (arrows), corresponding to (D) infarct size.

**Figure 2.** Flow–function relation in Group 1 dogs. Red denotes the infarcted zone, green the collateral blood flow–supplied zone, and blue the remote normal myocardium. MBF = myocardial blood flow; WT = wall thickening.
suggests that MBF alone cannot explain %WT within all the regions. The reduced WT despite relatively normal MBF was seen in only two of six dogs (seven stages) and indicates myocardial tethering. When these interspersed regions were excluded, all data points were described by a single relation ($r = 0.78$) (Fig. 6B).

**Comparisons between groups.** Table 2 illustrates pertinent results for a comparison between the Group 2 and 3 dogs. The heart rate, mean aortic pressure, and double product were not different between the two groups at both rest and peak dobutamine dose, although as expected, both the heart rate and double product were higher at stress within each group. The pressure gradients across the stenoses were similar in both groups of dogs as was the resting coronary blood flow. Although dobutamine increased coronary blood flow significantly more in the normal bed in the Group 2 dogs, it induced a similar degree of hyperemia in the arteries with stenoses in both groups. The MBF in the center of the stenosis-supplied beds was similar in both groups of dogs ($1.5 \pm 0.6$ vs. $1.3 \pm 0.4 \text{mL}\text{min}^{-1}\text{g}^{-1}$).

However, it was higher in the lateral collateralized portions in dogs with single-vessel compared with multi-vessel stenosis ($2.5 \pm 0.9$ vs. $1.8 \pm 0.4 \text{mL}\text{min}^{-1}\text{g}^{-1}$, $p < 0.001$).

**Figure 4.** Flow-function relation in Group 2 dogs. Red denotes the central portion of the perfusion defect corresponding to hypoperfused zone in panel C in Figure 3. Green denotes the collateral blood flow-supplied zone within the perfusion defect (that seen in lateral portions of the perfusion defect in panel A in Figure 3). Blue denotes the normal remote myocardium. MBF = myocardial blood flow; WT = wall thickening.
fitted to all data. This relation predicts that maximal WT cannot be >57% and that wall thinning will occur at MBF below 20% of normal. It also shows that there is no increase in WT when MBF exceeds three times normal.

**DISCUSSION**

The new information from this study is that the apparent disparity between the circumferential extents of abnormal WT versus IS at rest or between the circumferential extent of abnormal WT and PD size during DI can be explained largely by the level of CollBF in the regions with apparent WT abnormalities. The CollBF from adjacent normal beds provide intermediate levels of MBF to the lateral borders of IZ that determine the magnitude of WT in these regions. During myocardial infarction, although the reduction in MBF to the margins of the RA supplied by collaterals is adequate to prevent necrosis, it cannot preserve normal resting WT. Thus, in this setting, the extent of abnormal WT overestimates IS. Conversely, during DI, when MBF increases overall, CollBF at the lateral margins of the hypoperfused territory, although approximately 25% lower than MBF to normal regions, is adequate to preserve near normal WT. Therefore, the apparent extent of abnormal WT underestimates the IZ size during DI on visual inspection. Wall thickening and MBF mismatch is infrequent and occurs when ischemia spares small regions of the myocardium interspersed between larger IZs in the setting of multi-vessel stenosis. It is only in these rare instances that myocardial tethering plays a role in overestimating the extent of actual ischemia. Therefore, for all practical purposes, the MBF-WT relationship in acute ischemia remains inviolate.

**Acute myocardial infarction or ischemia at rest.** Infarction occurs only in myocardial regions with severely reduced MBF (<25% of normal) (17–19), whereas abnormal WT extends to regions beyond the infarction supplied by CollBF that is lower than MBF to the normal myocardium. These collateral-dependent regions are also ischemic and consequently exhibit reduced WT. The reduction in WT, however, parallels the reduction in MBF. Thus, the disparity between the extent of abnormal WT and infarction is not due to any mechanical factors as suggested previously (1–3), but simply due to gradations in MBF between the infarcted and normal beds.
Whereas the RA defines regions with reduced compared with normal MBF, it cannot be used to define gradations in MBF within the IZ (20). The center of the RA has very low MBF, whereas the lateral zones and epicardium have higher CollBF-derived MBF. In this study, we used MCE to show that regions with the RA supplied by CollBF exhibit intermediate MBF and WT values, but that these values are closely coupled similar to the regions with reduced and normal MBF.

**Figure 6.** Flow-function relation in Group 3 dogs when (A) all myocardial regions are included and (B) small segments in the anterior wall supplied by the diagonal branch interspersed between two large perfusion defects are excluded. Red denotes the central portions of the perfusion defects, and green and blue denote the collateral blood flow-supplied zones and the remote normal myocardium, respectively. MBF = myocardial blood flow; WT = wall thickening.

**Demand ischemia.** One of the advantages of assessing myocardial perfusion rather than function during DI is that PD precede WT abnormalities (4,21). Another reason is that even when perfusion and function are both abnormal, the extent of PD is usually greater than that of abnormal WT, resulting in easier detection of coronary stenosis by perfusion imaging, especially in single-vessel disease (4). Our study indicates that marked reduction in WT (compared with the normal bed) during stress occurs only within the central region of the PD. In the lateral margins, where MBF is higher because of collaterals, WT is reduced to a lesser degree and its magnitude depends on that of the CollBF.

In single-vessel stenosis, where CollBF is not compromised, the relationship between regional MBF and WT remains consistent during DI. This is predominantly the case in multi-vessel stenosis as well, except that CollBF is less in this setting and therefore the extent of abnormal WT is larger. In some instances, small regions with relatively normal MBF (for the level of stress) are interspersed between larger beds supplied by vessels with stenoses. These regions demonstrate WT that is significantly less than the level of MBF probably from myocardial tethering. The

**Table 2.** Selected Results From Groups 2 and 3 Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate at rest (beats·min⁻¹)</td>
<td>114 ± 19</td>
<td>129 ± 11</td>
</tr>
<tr>
<td>Heart rate at peak dobutamine dose</td>
<td>163 ± 25‡</td>
<td>182 ± 14‡</td>
</tr>
<tr>
<td>Aortic pressure* at rest (mm Hg)</td>
<td>87 ± 10</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>Aortic pressure at peak dobutamine dose</td>
<td>88 ± 11</td>
<td>97 ± 16</td>
</tr>
<tr>
<td>Double product† at rest</td>
<td>100 ± 20</td>
<td>126 ± 30</td>
</tr>
<tr>
<td>Double product at peak dobutamine dose</td>
<td>143 ± 27‡</td>
<td>183 ± 32‡</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Bed</th>
<th>Bed With Stenosis</th>
<th>Beds With Stenoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure gradient across stenosis (mm Hg)</td>
<td>—</td>
<td>17 ± 7</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Epicardial coronary blood flow at rest (mL·min⁻¹)</td>
<td>31 ± 10</td>
<td>33 ± 13</td>
<td>28 ± 13</td>
</tr>
<tr>
<td>Epicardial coronary blood flow at peak dobutamine dose (mL·min⁻¹)</td>
<td>94 ± 32‡</td>
<td>60 ± 32‡</td>
<td>58 ± 30‡</td>
</tr>
</tbody>
</table>

*Mean. †Heart rate × mean aortic pressure/100. ‡p < 0.001 compared with rest.
combination of tethering as well as reduced CollBF may explain the higher sensitivity of regional function assessment for detection of multi-vessel compared with single-vessel coronary stenosis during stress (4,22,23).

Clinical implications. Normal dogs have variable amounts of CollBF, which is more than in normal humans. However, patients with coronary artery disease can have extensive CollBF. Unfortunately, coronary angiography markedly underestimates collateralization in patients with coronary artery disease for several reasons. Most collateral connections are within the myocardium and are <100 μm in diameter, which is well below the spatial resolution of angiography (24). Epicardial connections commonly seen on coronary angiography do not provide an assessment of the spatial extent and magnitude of CollBF to the tissue (25,26). Finally, coronary collaterals (and in fact CollBF) can only be evaluated when there is a significant pressure gradient between the arteries supplying various myocardial regions. Although this occurs at rest frequently in patients with acute coronary syndromes (total or subtotal occlusion of a coronary artery), it is not that frequent in chronic coronary artery disease. To assess CollBF in chronic coronary artery disease without coronary occlusion, it is therefore necessary to induce a gradient between myocardial beds, which can be done by inducing coronary hyperemia as shown in this study.

Our findings were made in the setting of a previously normal LV. In the situation with previous infarction or reduced resting MBF, LV remodeling and increased wall stress are noted even in regions with normal MBF (27). Thus, %WT may be abnormal in these regions not only at rest but also during DI despite a normal MBF response to increased myocardial oxygen consumption. Our results would generally not be relevant in that situation.

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